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FINAL TECHNICAL REPORT

**Title: Molecular Probes in Marine Ecology: Concepts,
Techniques and Applications**

**Office of Naval Research
Program: Advanced Training in Molecular Marine Biology**

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Grant Number N00014-89-J-1933

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**Principal Investigator and Course Director: J. Woodland Hastings
Professor of Biology, Harvard University**

Institution: Marine Biological Laboratory

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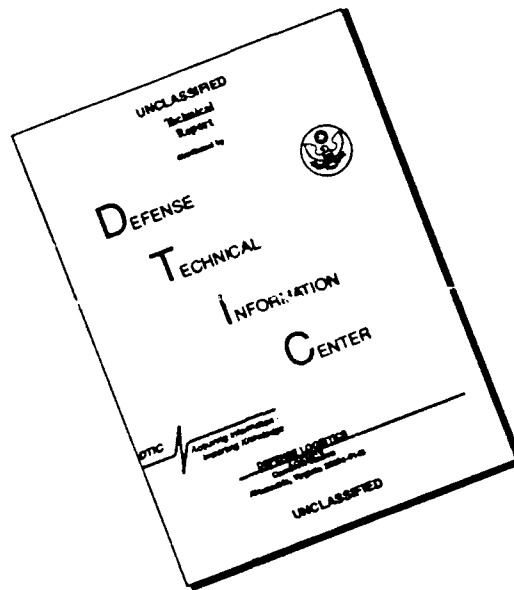
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L OBJECTIVES OF THE COURSE

A. Philosophy: Ecology interfaces with and depends upon many scientific disciplines, ranging from geophysics and atmospheric chemistry to population genetics and behavioral biology, to name just a few. Likewise, ecological issues touch all of humanity, with concerns ranging from sewage disposal and recycling to the ozone hole and the greenhouse effect. Again, the actual list is a much longer one, and the concerns are compelling.

How can scientists address and provide guidance in the solution of such problems? There is we think, no single or simple approach. Many significant steps have already been taken, involving many scientists and many initiatives. Among these, the investigation and understanding of marine ecosystems can be viewed as one of the most important. By virtue of its buffering capacity for many physical and chemical variables, and because of its biological productivity, the ocean is central to both the realities of the issues and the understanding of the problems. Ecology, in whatever disguise, embraces the many questions and seeks workable solutions. The 1990 MBL marine ecology course, entered into its second year of a program in molecular marine biology, providing training in the use of molecular probes for ecological problems. At the same time, it attempted to formulate a conceptual framework as to how to approach the more global ecological issues.

B. History and Tradition: The Marine Ecology course at MBL was introduced in 1956 and has been offered each summer since that time. In more recent years its interaction and cooperation with the MBL year-round Marine Ecosystems program has been significant.

C. Training Goals: During the past decade, developments in the area of molecular biology, biotechnology, immunology and microsensing techniques have revolutionized many areas of biological sciences. We designed this course for graduate students, postdoctoral fellows and established investigators who wish to learn these several techniques and their applications to physiological and ecological investigations.

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A-1	

II. COURSE ORGANIZATION

A. Structure of the Course: The six week course was organized around three three-week long modules offered simultaneously and repeated a second time. The students thus rotated through two of the three laboratory sections offered concurrently in two cycles.

The first module was under the direction of Dr. T.T. Chen assisted by Dr. Chun Min Lin and Clara Cheng. The second module was under the direction of Dr. Dennis Powers, assisted by Dr. Lynna Hereford, Toby Cole and Simona Sorger. The third module under the direction of Dr. Kenneth Nealson, assisted by Dr. Charles Wimpee and Keno Trüper. Dr. John Hobbie acted as the organizer for the mini-symposium on trace gases and global warming; Ann Bucklin served as a student seminar coordinator and Barbara Wimpee was the head course assistant.

B. Students: Twenty-four students (see Appendix) were accepted in the course, the number being based on laboratory layout and facilities. This is certainly a maximum number for the faculty to handle in the type of instruction given.

In selecting students we attempted to achieve diversity in as many ways as possible, ranging from specific interests and educational background to career stage and geographical location. Applications from minorities were solicited and given special attention. However, the small numbers involved make it difficult to assess minority participation.

Because of the advanced nature of the course, and its specific applicability to students' research problems at home institutions, no undergraduates were among those accepted in the course. The participants included thirteen graduate students, six at the postdoctoral level and five faculty members. An additional student was a member of the science writing program.

C. Lectures and Seminars:

(1) Daily: Lectures by staff and special visitors were given 6 days per week, usually at 0900, with some additional lectures on certain days. The speakers and titles are given in the appendix. Outside speakers are also listed in the appendix.

(2) Student Seminars: A special and very successful feature of this course was the student seminar program. Organized and led by Dr. Ann Bucklin, each of the students presented a research seminar to the group. These were held at 8PM on two evenings per week. This allowed students to discuss the applicability of techniques learned in

the course to their individual research projects. Titles and speakers are given in the appendix.

D. Mini-Symposia: The course included three mini-symposia. Two of these were cosponsored with the microbiology course, the first of these being on bioluminescence and symbiosis on July 7, and the second on molecular approaches to microbial ecology on July 21. A final three-day mini-symposium on global ecological issues was convened by Dr. John Hobbie, it was entitled "Trace Gases and Global Warming".

III. DESCRIPTION OF THE COURSE

A. Overview: The lectures and laboratories introduced participants to the theory and practice of several key techniques in molecular biology, and their application to the study of important ecological problems.

B. Laboratory:

The first module, under the direction of Dr. T.T. Chen, was concerned with cloning, manipulation and analysis of nucleic acid probes. Using fish and other marine organisms as experimental material, nucleic acids were isolated, characterized and used in the construction of specific probes. Students utilized a variety of procedures and techniques, including purification and quantitation of RNA and DNA; dot blots, electrophoresis, Northern and Southern blotting and restriction mapping; construction of cDNA libraries; oligonucleotide synthesis and the creation of synthetic probes; and screening cDNA libraries and DNA sequencing.

The second module, under the direction of Dr. Dennis Powers, was concerned with the analysis of population and species with nucleic acid probes. These exercises capitalized on the use of mitochondrial DNA, chloroplast DNA and DNA fingerprinting, along with the amplification of 16S-RNA and other specific genes, to address questions of genetic variability within and between marine species and populations. The laboratory techniques included isolation, purification, and restriction analysis of DNA; oligonucleotide synthesis, Southern blotting and *in vitro* gene amplification by the polymerase chain reaction; and DNA sequencing.

The third module, under the direction of Dr. Kenneth Nealson, was concerned with symbioses, including the identification of organisms and their functional interrelationships. Biochemical techniques, including enzyme assays, were complemented with immunochemical analysis including Western Blots. The use of

specific nucleic acid probes involved Southern and Northern blotting as well as PCR amplification. Symbioses examined included those that contribute energy for photosynthesis and sulfur oxidation, carbon fixation, nitrogen fixation, and bioluminescence.

Acknowledgments: MBL personnel in many different departments were cooperative and helpful. We wish to acknowledge in particular the help of Les Garrick and Florence Dwane in organizing the course and registering students, and Linda Huffer in obtaining equipment on loan. Funding for the course was provided in part by a contract from the Office of Naval Research and by a grant from the Merck Sharp & Dohme Research Laboratories.

Appendices

- A. Staff of 1990 Marine Ecology Course**
- B. Students enrolled in the 1990 Marine Ecology Course**
- C. Lecture schedules, 1990 Marine Ecology Course**
- D. List of student seminars, 1990 Marine Ecology Course**
- E. Mini-Symposium "Trace Gases and Global Warming"**
- F. Laboratory Exercises 1990**
- G. 1990 Course Announcements**
- H. List of loaned equipment, 1990**

Appendix A

Marine Biological Laboratory Molecular Probes in Marine Ecology: Concepts, Techniques and Applications June 17 - July 28, 1990

Faculty (Continued):

Barbara Wimpee, Course Coordinator
Great Lakes Research Center
University of Wisconsin-Milwaukee

Charles Wimpee, Instructor
University of Wisconsin-Milwaukee

Lecturers:

David Caron
Colleen Cavanaugh
Penny Chisholm, Woods Hole Oceanographic Inst.
Ed De Long, Woods Hole Oceanographic Inst.
Paul Dunlap, Woods Hole Oceanographic
Brian Fry, Marine Biological Laboratory
Linda Goff
John Kessler
Lynn Margulis, University of Massachusetts, Amherst
James McCarthy
Dan Morse
Rob Olsen, Woods Hole Oceanographic Inst.
Hans Paerl
Jack Palmer
Ned Ruby
G. Saylor
Ann Sesholz
Bob Simon
Mitch Sogin, Marine Biological Laboratory
Felix Strumwasser, Marine Biological Laboratory
John Waterbury, Woods Hole Oceanographic Inst.

Marine Biological Laboratory
Molecular Probes in Marine Ecology: Concepts, Techniques and Applications
1990 Accepted Students

Abdiel J. Alvarez, Graduate
University of Puerto Rico

Brian J. Binder, Post-Doctoral
Massachusetts Institute of Technology

*Alice F. Brown, Graduate
Brown University

Ka Hou Chu, Faculty
Chinese University of Hong Kong

Peter J. Edmunds, Post-Doctoral
Northeastern University

Oivind Enger, Graduate
University of Bergen, NORWAY

*Jonathan B. Geller, Post-Doctoral
University of Oregon

*Gregory J. Hinkle, Graduate
University of Massachusetts, Amherst

Robert E. Hodson, Faculty
University of Georgia

*Eric R. Holm, Graduate
Duke University

Jerilyn Jewett-Smith, Post-Doctoral
Whitman College

*Lisa M. Kann, Graduate
University of Rhode Island

James S. Maki, Post-Doctoral
Harvard University

*Kirk D. Malloy, Graduate
University of Delaware

*Adam G. Marsh, Post-Doctoral
University of New Hampshire

Appendix C.
Lecture Schedule

Marine Ecology Lecture Schedule /MBL Summer '90
All Lectures 9 AM Homestead Lecture Hall

Week 1

June

- 18 Mon. Woody Hastings: Bioluminescence in the marine environment: an ecological enigma. Who has the genes, where do they come from, and what are they for?
- 19 Tues. Kenneth Nealson:
Woody Hastings: Biochemistry and physiology of bacterial bioluminescence.
- 20 Wed. Paul Dunlap: The lux genes in bacteria: organization, structure, and expression.
- 21 Thurs. Chuck Wimpee: Development and use of species-specific *luxA* gene probes for identification of field collected bacteria.
- 22 Fri. Dennis Powers: Population genetics of the teleost *Fundulus heteroclitus*:
- 23 Sat. Dennis Powers: Adapting to a changing environment: molecular and genetic mechanisms.

Week 2

June

- 25 Mon. Tom Chen: Evolution of growth hormone genes in teleosts and molluscs.
- 26 Tues. Tom Chen: Molecular toxicology: an approach to study sublethal levels of environmental pollutants.
- 27 Wed. Chuck Wimpee: Use of the plastid RBC-L gene sequence for investigating the phylogeny of primitive angiosperms.
- 28 Thurs. Chuck Wimpee: Abandoning photosynthesis: the molecular consequences in parasitic plants.
- 29 Fri. Bob Simon: Biology of the air-water interface
- 30 Sat. Dan Morse: Molecular mechanisms controlling metamorphosis of marine invertebrate larvae: signals, receptors, transducers & genes.

July 10, 1990

Marine Ecology Lecture Schedule /MBL Summer '90
All Lectures 9 AM Homestead Lecture Hall

Week 3

July

- | | | |
|----------|---|--|
| 2 Mon. | C.M. Lin: | Sequencing of DNA and RNA |
| 3 Tues. | Field Trip: | John Valois with MBL & WHOI staff scientists. |
| 4 Wed. | | Holiday |
| 5 Thurs. | James McCarthy | Physical and chemical factors regulating the productivity of marine plankton: why are the determinations so difficult? |
| 6 Fri. | John Waterbury:
Rob Olsen: | The physiological ecology of marine <i>Synechococcus</i> .
Analysis of marine picoplankton using flow cytometry. |
| 2 PM: | Rob Olsen: | Laboratory demonstration of flow cytometry |
| 4 PM: | John Kessler: | Path and pattern: The mutual dynamics of swimming eucaryotic microorganisms and their environment. |
| 7 Sat. | Ken Nealson:
Ned Ruby:
Paul Dunlap: | Bioluminescence and Symbiosis
(Minisymposium Co-Sponsored with Microbiology) |

Week 4

July

- | | | |
|-----------|--------------------|---|
| 9 Mon. | Ed De Long: | Use of fluorescently labeled ribosomal mRNA probes probes for microbial identification. |
| | Ken Nealson: | Taxonomy physiology and distribution of marine bioluminescent bacteria. |
| 10 Tues. | Ann Bucklin: | Allozymic and mtDNA indicators of population genetic structure and dispersal in marine organisms. |
| 11 Wed. | Mitch Sogin: | Unexpected genetic diversity in the eucaryotic kingdom. |
| 12 Thurs. | Colleen Cavanaugh: | Intracellular chemoautotrophic symbionts in molluscs. |
| 13 Fri. | Tom Chen: | Applications of gene transfer technology to marine ecology. |
| 14 Sat. | Lynn Margulis: | Symbiogenesis and symbiontism |

July 10, 1990

Marine Ecology Lecture Schedule /MBL Summer '90
All Lectures 9 AM Homestead Lecture Hall

Week 5**July**

- 16 Mon. *Penny Chisholm:* Cell cycle regulation in pro and eucaryotic marine phytoplankton.
- 17 Tues. *Woody Hastings:* Circadian rhythms in marine dinoflagellates: cellular and molecular processes.
- 18 Wed. *Felix Strumwasser:* A neuronal circadian oscillator in the marine mollusc, *Aplysia*.
- 19 Thurs. *Jack Palmer:* Tidal and lunar rhythms in shore dwelling animals.
- 20 Fri. *John Hobbie:* Anaerobic decomposition processes in a coastal system.
- 21 Sat. *Ed DeLong:* Molecular Approaches to Microbial Ecology
G. Saylor: (Minisymposium Sponsored by Microbiology)
D. Lane:

Week 6**July**

- 23 Mon. *Brian Fry:* Stable isotope studies of bacterial growth and nutrition in the Parker River Estuary, MA
- 24 Tues. *David Caron:* Role of small protozoa in plankton communities.
- 25 Wed. *Hans Paerl:* The applications of immunochemical and molecular tools for identification and characterization of marine nitrogen fixing assemblages.
- All day field trip to the great Sippewisset Marsh.**
- 26 Thurs. *Hans Paerl:* Field studies on nitrogen fixing communities. The importance of microenvironments in microbial biogeochemical transformations.
- 10:30 AM *Linda Goff:* Horizontal gene transfer in marine symbioses.
- Afternoon Mini-symposium on Global Ecological Issues: Trace Gases and Global Warming. *John Hobbie*, Convener.
- 27 Fri. Min-Symposium (continued)
All Day
- 28 Sat. Morning Mini-symposium (final session)

Appendix D.
Student Seminars

MARINE ECOLOGY COURSE - SUMMER 1990
STUDENT SEMINAR SERIES
Meetings: 8 pm, Loeb 3rd floor Conference Room

WEEK 1

Monday, 6/18

Greg Hinkle (Boston Univ.): Symbiosis and the origin of eukaryotic motility

Tuesday, 6/19

Jim Maki (Harvard Univ.): Adhesion of bacteria and barnacles

Wednesday, 6/20

Brian Binder (MIT): Combining flow cytometry with 16S rRNA-targetted probes: A potential new tool for microbial ecology.

Thursday, 6/21

Robin Schneider (Univ.S.Louisiana): Cloning and analysis of repetitive DNA in Plethodon cinereus, Menippe mercenaria, and M. adina.

WEEK 2

Tuesday, 6/26: Life Histories

Adam Marsh (Univ. New Hampshire): Gene control and life history strategies

Kirk Malloy (Univ. Delaware): Studies in recruitment success and population genetics of flounder.

Wednesday, 6/27: Bio-Fouling (etc.)

Eric Holm (Duke Univ.): Genetics of two attachment responses in the barnacle Balanus amphitrite.

Bob Sinsabaugh (Clarkson Univ.): Exoenzyme activities in lotic biofilms.

Bruce Jacobs: The Write Stuff: Science Journalism for Beginners

WEEK 3

Monday, 7/2: Microbial Ecology

Bob Hodson (Univ. of Georgia): Bacterial degradation of lignins

Abdiel Alvarez (Univ. Puerto Rico): Survival characteristics of a plasmid-containing and a non plasmid-containing E. coli strain under in situ conditions.

Thursday, 7/5: Symbiosis

Martin Polz (Univ. of Vienna): Nematodes and their ectosymbiotic bacteria

Jerilyn Jewett-Smith (Whitman College): Allelopathy in aquatic systems

Marine Ecology 1990: Student Seminar Series (Page 2)

WEEK 4

Monday, 7/9: Physiology

Ka Chu (Chinese Univ. of Hong Kong): Physiological studies on the early life-history stages of penaeid shrimp.

Michael Schmale (Univ. of Miami): Cancer in Damselfish: Who, What, Where, How, So What?

Wednesday, 7/11: Coral Reef Ecology

Peter Edmunds (Northeastern Univ.): Energy budgets of colonial marine invertebrates.

Eric Wommack (Univ. of Maryland): Coral reef microbial ecology.

WEEK 5

Tuesday, 7/17: Populations

Jon Geller (Univ. Oregon): Marine biological invasions.

Alice Brown (Brown Univ.): Dispersal and supralittoral copepods.

Wednesday, 7/18: Populations

Lisa Kann (Univ. of Rhode Island): Genetic structure of Calanus finmarchicus in the Gulf of Maine.

Stephen Tsoi (Univ. of Hong Kong): The lactate dehydrogenase isozymes in Anquilla japonica (Japanese eel).

WEEK 6

Monday, 7/23

Tracie-Lynn Nadeau (Univ. Wisc. - Milwaukee): Adaptation of PCR for studying specific micro-organisms in aquatic communities.

Lynda Shapiro (Bigelow Laboratory): Ultraplankton species: Who, Where, and Why (And How To Figure It Out).

Wednesday, 7/25: Parasitism

Oivind Enger (Univ. Bergen, Norway): Survival of fish pathogenic bacteria in the marine environment.

Erika Stephens (Harvard Univ.): A model system for looking at disease in natural populations.

Appendix E

MARINE BIOLOGICAL LABORATORY
WOODS HOLE, MA 02543

"Controls of Trace Gas Release to the Atmosphere"
A Minisymposium Sponsored By The MBL 1990 Marine Ecology Course
July 26 - 28, 1990

Dr. John Hobbie, Symposium Organizer
Ecosystems Center, MBL

Lecturers:

R. Houghton, Woods Hole Research Center
R. Bowden, Ecosystems Center, MBL
C. Goyet, Woods Hole Oceanographic Institution
P. Falkowski, Brookhaven National Laboratory
P. Groffman, University of Rhode Island
H. Hemond, Massachusetts Institute of Technology
M. Scranton, SUNY, Stony Brook
P. Crill, EOS, University of New Hampshire
James Tiedje, Center for Microbial Ecology, Michigan State University
P. Steudler, Ecosystems Center, MBL
J. Dacey, Woods Hole Oceanographic Institution

CONTROLS OF TRACE GAS RELEASE TO THE ATMOSPHERE

A MINISYMPOSIUM SPONSORED BY THE MBL 1990 MARINE ECOLOGY COURSE HOMESTEAD BUILDING, MBL

- | | | |
|----------------|--------------|---|
| 26 July | 1:00 | Welcome and introductory material. J.Hobbie (MBL) |
| | 1:30 | Controls of carbon dioxide fluxes by land use changes
R.Houghton (Woods Hole Research Center) |
| | 2:45 | Controls of CO₂ and N₂O fluxes in forests
R.Bowden (Ecosystems Center, MBL) |
| | 4:00 | Physical/chemical controls of CO₂ in the ocean
C.Goyet (Woods Hole Oceanographic Institution) |
| 27 July | 9:00 | Biological cycles and CO₂ in the ocean
P.Falkowski (Brookhaven National Laboratory) |
| | 10:30 | Trace gases from agricultural lands
P. Groffman (URI) |
| | 1:30 | Controls of methane release from bogs
H.Hemond (MIT) |
| | 2:45 | Methane cycling in the ocean
M.Scranton (SUNY Stony Brook) |
| | 4:00 | Controls of methane release from wetlands
P.Crill (EOS, Univ. of New Hampshire) |
| | 8:00 | Friday night lecture by James Tiedje, Director,
Center for Microbial Ecology, Michigan State Univ. |
| 28 July | 9:00 | Controls of methane and sulfur gas flux in forests
P.Steudler (Ecosystems Center, MBL) |
| | 10:30 | Sulfur gas flux from the ocean
J.Dacey (Woods Hole Oceanographic Institution) |

Appendix F.
Laboratory Outlines: Modules 1, 2 & 3

MODULE I PROPOSED LECTURE OUTLINES

by T.T. Chen and C.M.Lin

I. Introduction:

- Important discoveries in Molecular Biology leading to recombinant technology
- Differences of cDNA and genomic sequences
- Why is it necessary to clone cDNA and genomic sequences ?

II. Basic Nucleic Acid Methodology

- Preparation of DNA and RNA
- Important enzymes for manipulation of DNA and RNA molecules
- PCR amplification of DNA molecules
- Southern blot vs northern RNA blot analysis

III. Construction of cDNA and Genomic Libraries

- General consideration of constructing cDNA and genomic libraries
- Basic strategy of cloning cDNA of abundant mRNA species
- Basic strategy of cloning cDNA of rare mRNA species
- Genomic subtraction for cloning DNA corresponding to deletion mutations or sex specific sequences
- Application of PCR in cDNA or DNA cloning

IV. Screening and Characterization of Recombinant Clones

- Screening of clones by nucleic acid hybridization
- Screening of clones by immunochemical reactions
- Screening of clones by specific characteristics of gene products
- Restriction enzyme analysis and subcloning

V. General Consideration of Designing and Application of Nucleic Probes

VI. Nucleic Acid Sequence Determination

- Preparation of templates
- Sequencing reaction (single strand and double strand templates)
- Gel electrophoresis and data interpretation
- Sequencing of RNA template
- Trouble shooting

MODULE I LABORATORY SCHEDULE

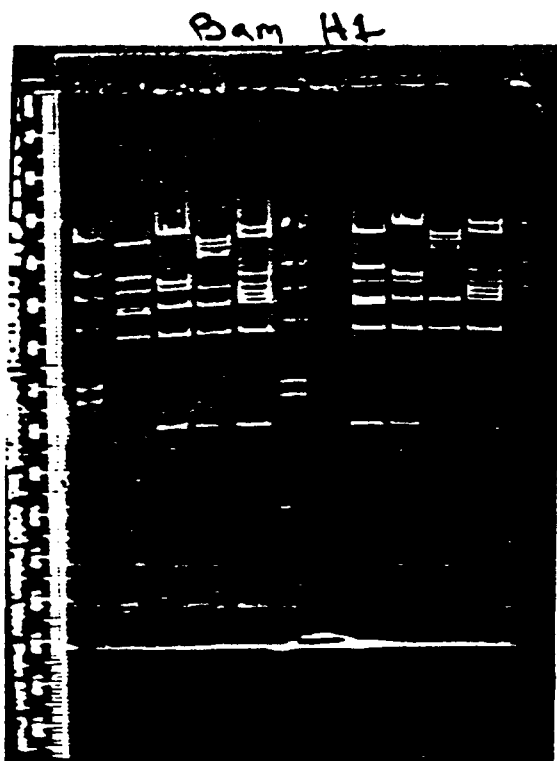
Date	Topics	By T.T. Chen
6/18 (Mon)	Preparation of total RNA	
6/19 (Tus)	Preparation of poly(A) ⁺ -RNA, gel electrophoresis and transfer of RNA to nitrocellulose membranes	
6/20 (Wed)	Synthesis of 1st and 2nd-strand cDNA; sizing ds-cDNA by electrophoresis	
6/21 (Thu)	PCR amplification of ds-cDNA; methylation and linker ligation (o/n))	
6/22 (Fri)	Trimming off excess linkers; ligation of ds-cDNA to gt-10 arms (o/n)	
6/23 (Sat)	Packaging and titration of the library	
6/24 (Sun)	Plating out library for screening	
6/25 (Mon)	Blotting, prehybridization and hybridization; labelling probes	
6/26 (Tus)	Washing membranes and autoradiography; <i>preparation of plasmid and phage DNA</i>	
6/27 (Wed)	Developing X-ray film, isolating positive plaques and plating out for 2nd screening; <i>restriction enzyme digestion and gel electrophoresis</i>	
6/28 (Thu)	Blotting, prehybridization and hybridization; labelling probes; <i>transferring DNA fragments to nitrocellulose membranes</i>	
6/29 (Fri)	Washing membranes and autoradiography; <i>labelling probes, prehybridization and hybridization</i>	
6/30 (Sat)	Developing X-ray film and isolating positive plaques; <i>washing blots and autoradiography</i>	
7/1 (Sun)	<i>Developing X-ray film</i>	

MODULE I LABORATORY SCHEDULE

Date	Topics	By T.T. Chen
7/9 (Mon)	Preparation of total RNA	
7/10 (Tus)	Preparation of poly(A) ⁺ -RNA, gel electrophoresis and transfer of RNA to nitrocellulose membranes	
7/11 (Wed)	Synthesis of 1st and 2nd-strand cDNA; sizing ds-cDNA by electrophoresis	
7/12 (Thu)	PCR amplification of ds-cDNA ; methylation and linker ligation (o/n)	
7/13 (Fri)	Trimming off excess linkers; ligation of ds-cDNA to gt-10 arms (o/n)	
7/14 (Sat)	Packaging and titration of the library	
7/15 (Sun)	Plating out library for screening	
7/16 (Mon)	Blotting, prehybridization and hybridization; labelling probes	
7/17 (Tus)	Washing membranes and autoradiography; <i>preparation of plasmid and phage DNA</i>	
7/18 (Wed)	Developing X-ray film, isolating positive plaques and plating out for 2nd screening; <i>restriction enzyme digestion and gel electrophoresis</i>	
7/19 (Thu)	Blotting, pre hybridization and hybridization; labelling probes; <i>transferring DNA fragments to nitrocellulose membranes</i>	
7/20 (Fri)	Washing membranes and autoradiography; <i>labelling probes, prehybridization and hybridization</i>	
7/21 (Sat)	Developing X-ray film and isolating positive plaques; <i>washing blots and autoradiography</i>	
7/22 (Sun)	<i>Developing X-ray film</i>	

PCR hybridization

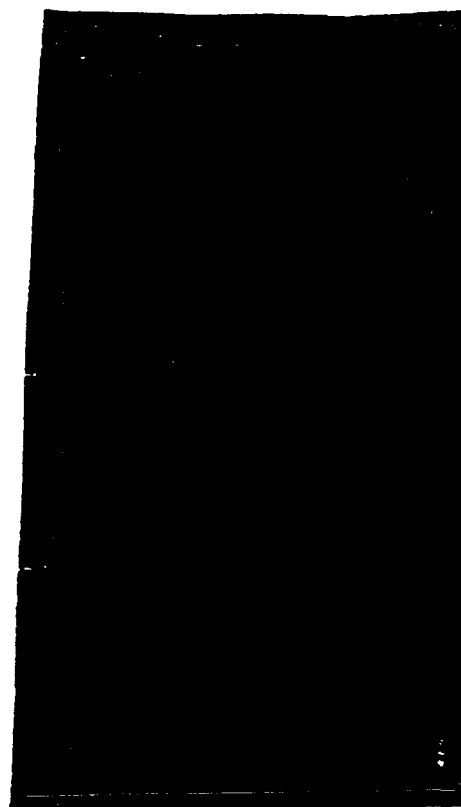
Fig 3



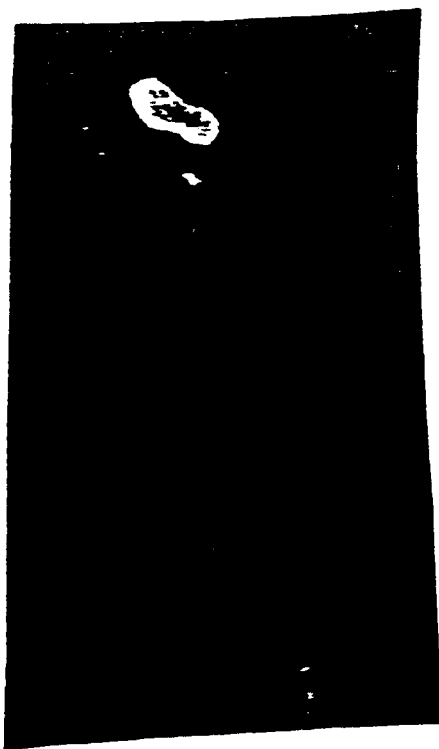
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(a)

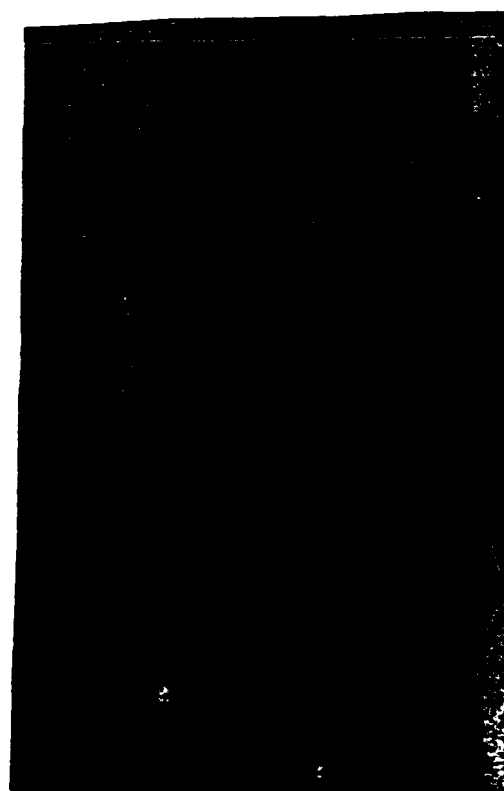
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(b)

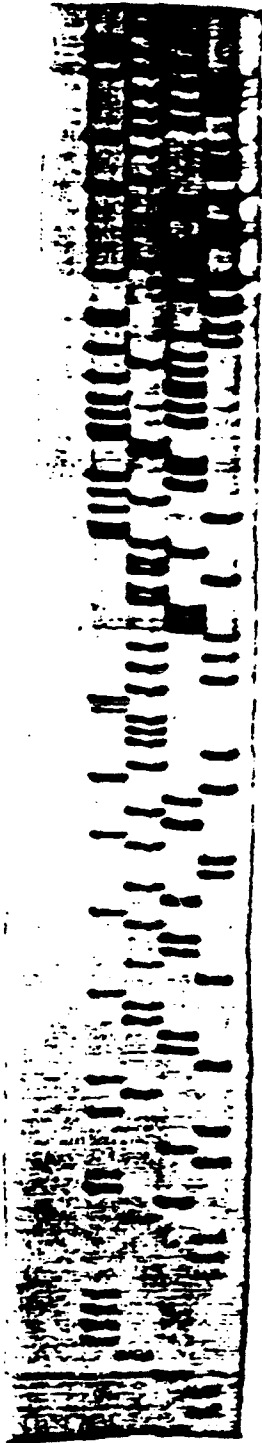


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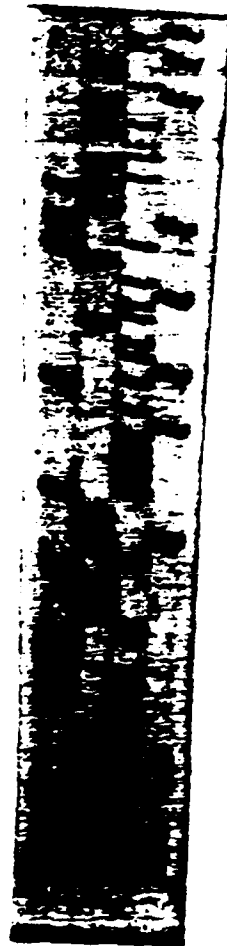


(d)

Fig 4

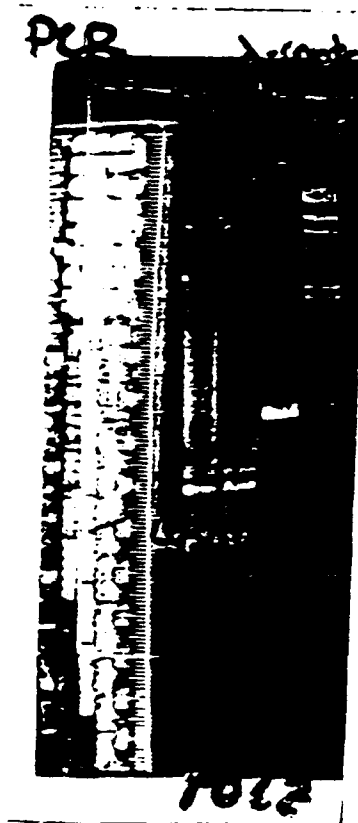


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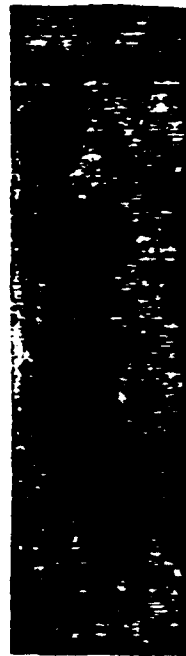


(b)

Fig 5



(a)



(b)

RESULTS FROM THE SESSION TWO OF MODULE I

Fig 1. Poly-A⁺ RNA isolated from (a) shark and (b) skate.

Fig 2. Double-stranded cDNA prepared from poly-A⁺ RNA. (a) ethidium bromide stained cDNA (b) autoradiograph of cDNA.

Fig 3. Four different RTVG clone DNAs were digested with (a) Eco RI (b) Bam HI (c) Eco RI and Bam HI restriction enzymes and transferred to nylon membranes, then hybridized to pRTC2 probe which contains the cDNA of rainbow trout vitellogenin gene.

Fig 4. DNA sequencing using (a) single strand DNA and (b) double strand DNA. (see Session one Fig 4)

Fig 5. PCR products from the amplification of lambda gt 10 cDNA library using IGF related primers. (a) total PCR products (b) hybridization of IGF related primer to the PCR products.

Fig 6. The screening of cDNA library with IGF-like probe.

(a)

(b)

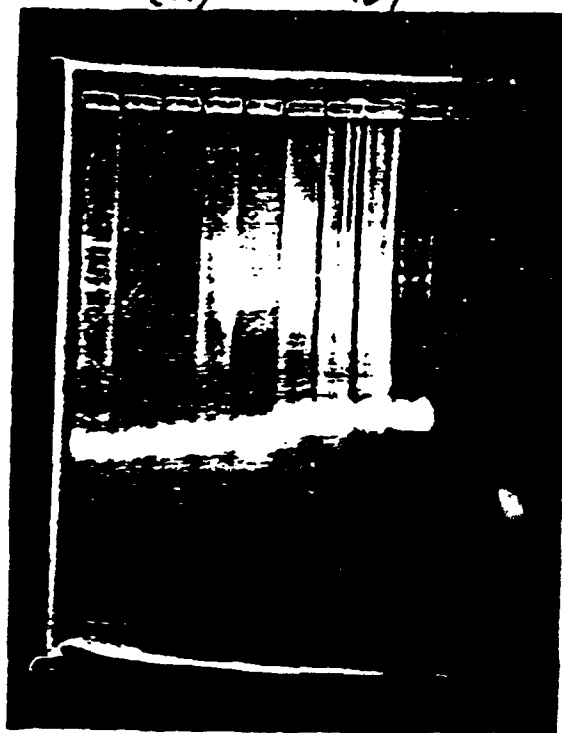


Fig 1

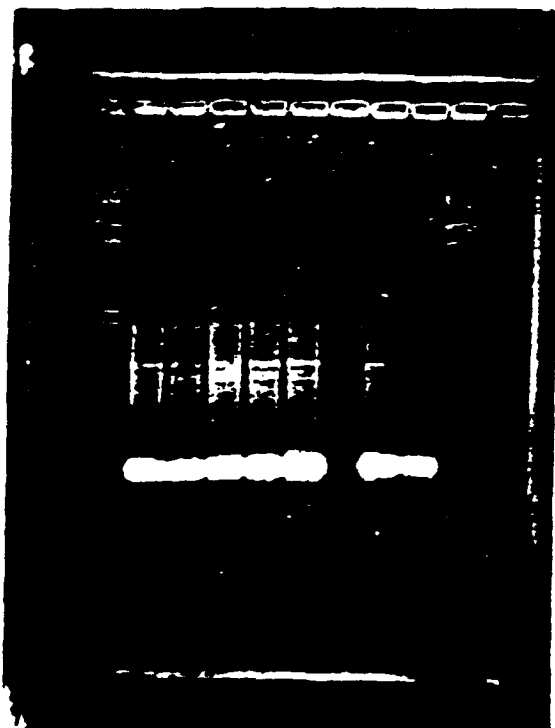


Fig 2 (a)

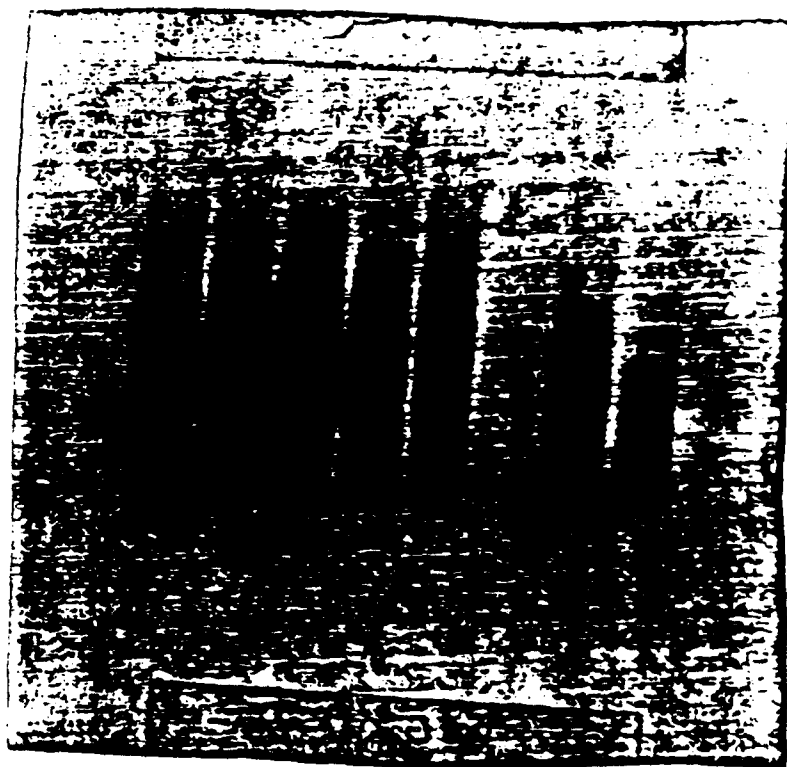


Fig 2 (b)

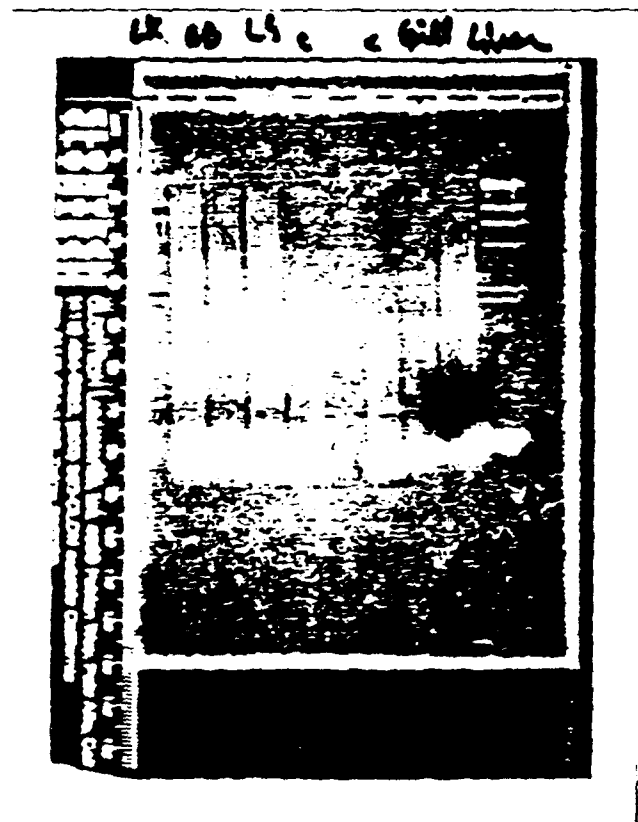
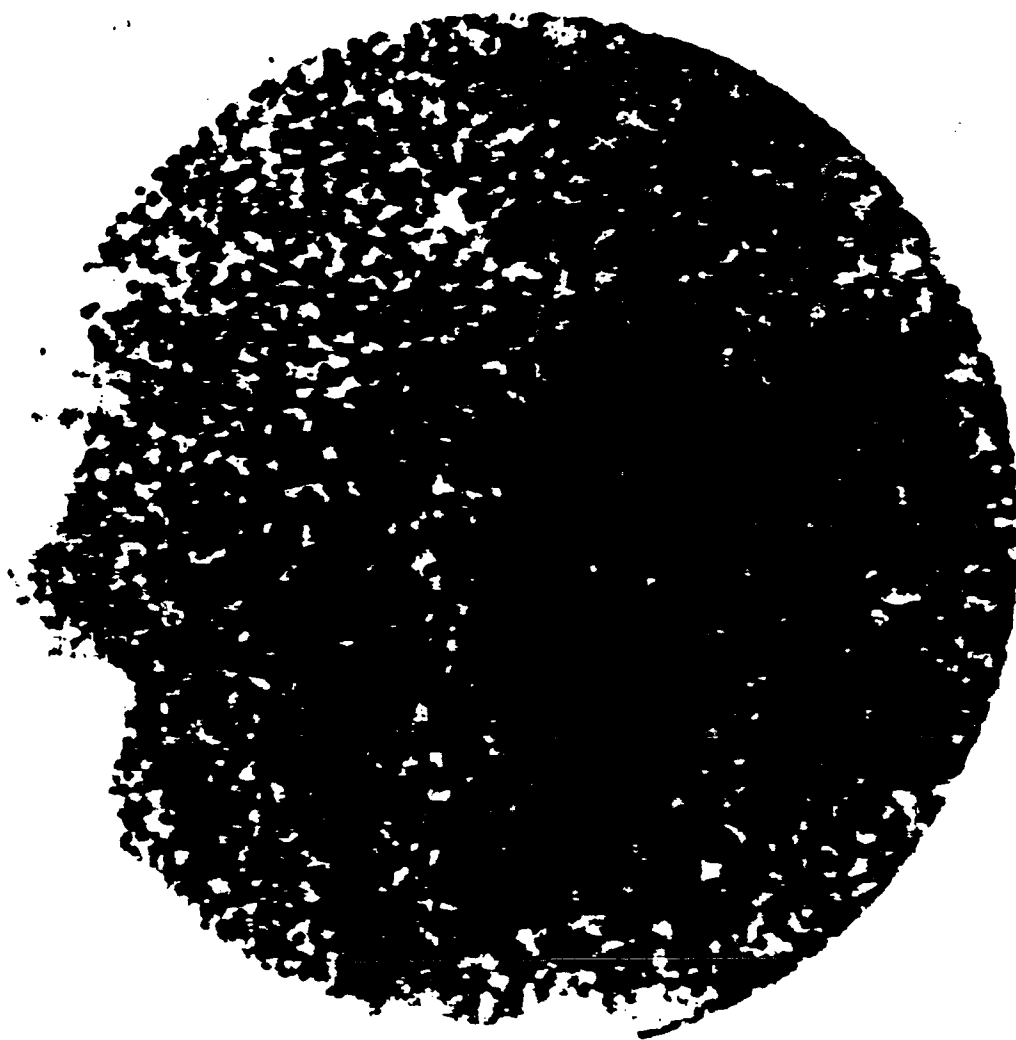


Fig 5 (a)

Fig 5 (b)

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Appendix F.
Laboratory Outlines: Modules 1, 2 & 3

MODULE 2

POWERS/HEREFORD/COLE/SORGER

Analysis of intraspecific mitochondrial DNA restriction
length polymorphisms using Southern blotting and PCR

Day 1:

Field trip to collect fish (picnic lunch generously
provided by Cafe Swope)
Isolation and restriction enzyme digestion of mitochondrial
DNA
Isolation of DNA from single eggs for PCR

Day 2:

Southern Transfer
Random prime probe
Finish purification of DNA from single eggs

Day 3:

Hybridization of blot
Set up PCR using 12 and 16s mitochondrial DNA primers

Day 4:

Wash up and autoradiograph Southern blot
Analyze PCR products by gel electrophoresis
Restriction enzyme digestion of PCR products

Day 5:

Develop Southern blots
Purification of PCR fragments for sequencing

Day 6:

PCR lecture
Discussion of results from Southern and PCR

Day 7:

Go to the beach

Analysis of mitochondrial DNA polymorphisms by DNA
sequencing.

Day 8:

Double and single stranded DNA sequencing

Individual projects

Days 9-14:

Design projects based on techniques learned during first
part of session

Mitochondrial DNA isolation

The following laboratory steps were derived from two published procedures (Chapman and Powers, 1984, and Gonzalez-Villasenor et al., 1986)

Isolation of mtDNA

The best tissues for mitochondria isolation are eggs and ovaries. Soft digestive tract tissue is also a reasonable source of mitochondria. Samples must be kept at 4 C (on ice) until organic extraction steps.

TEK Buffer:

100 ml:

50 mM Tris-Cl

10 mM EDTA

1.5% KCl

10 ml 0.5 M Tris-Cl (pH=7.5)

2 ml 0.5 M EDTA (pH=8.0)

20 ml 1.0 M KCl

Important: Keep everything ice cold until organic extraction.

1. Dissect fish - transfer ovaries to a 7 ml Dounce Homogenizer (B). Add 2 ml of ice cold TEK Buffer and grind 15-20 times.
2. Increase volume to 15 ml with ice cold TEK and agitate into solution.
3. Transfer to 30 ml tube and spin 20 minutes at 3K rpm (1000g) in Sorvall at 4 C in a SS34 or SM24 rotor.
4. Transfer supernatant to a new tube leaving the pellet of nuclei and cell debris behind.
5. Spin 20 min. at 3K rpm in Sorvall. Repeat transfer of supernatant to new tube.
6. Spin 30 min. at 12.5K rpm (18,000g) in Sorvall at 4 C, to pellet mitochondria.
7. Pour off supernatant, resuspend pellet in 2 ml of ice cold TEK by vortexing.
8. Spin 30 min. at 12.5K rpm in Sorvall at 4 C. Pour off supernatant.

Extraction of DNA from mitochondria

1. Lyse mitochondria by adding 0.5 ml TEK with 5% NP-40. Vortex and let sit on ice for 10 min. Vortex again.
2. Spin 10 min. at 12.5K rpm, 4 C in Sorvall.

3. Transfer supernatant to a 1.5 ml eppendorf tube.
4. Add 0.5 ml. of equilibrated Phenol, vortex then spin for 5 min. in eppendorf centrifuge. Save supernatant (sup.) by transferring to a new tube.
5. Add 0.5 ml of Chloroform, vortex, spin for 5 min. Save sup., again, and transfer to new tube.
6. Add 1.0 ml. cold* 95% ethanol (EtOH), vortex and let sit at -80 C for 1 hour. Spin down precipitate (5 min. in eppendorf centrifuge) and pour off sup.
7. Wash with cold* 70% EtOH: Add 1.0 ml of cold 70% EtOH
Spin down precipitate (ppt.)
Pour off sup. carefully
Do not lose pellet
8. Dry down ppt. and resuspend in 80 ul of TE.
9. Run an aliquot of the DNA on a gel.

*note: 70% and 95% EtOH should be stored in -20 C freezer until ready to use.

ISOLATION OF DNA FROM SINGLE EGGS FOR PCR

1. Take a single egg and put in an eppendorf tube
2. Add 100 ul 0.1 M Tris (pH 8.0) 0.1 M NaCl
3. Mash egg with eppendorf grinder
4. Add 1 ul of Proteinase K (10 mg/ml) and incubate at 37 °C overnight
5. Add an equal volume of phenol/chloroform, vortex, then spin in eppendorf centrifuge for 5 minutes. Remove aqueous layer. Repeat using chloroform/isoamyl alcohol. Add 10 ul 3 M NaOAc and 200 ul ethanol. Vortex then put at -20 °C for 1 hour. Spin for 15 min in eppendorf in cold room. Decant and add 200 ul cold 70% ethanol. Spin in cold for 10 minutes. . Decant then dry in speed vac. Resuspend in 10 ul TE.

SOUTHERN TRANSFER and HYBRIDIZATION

1. Load digested DNA on a 0.8 % agarose gel. Run at 50V for approx. 4 hrs.
2. Shake in denaturing solution 2 X 15 min.
3. Rinse gel with dH2O and shake in neutralizing soln. for 30 min.
4. Set up blot. Cut nylon membrane to size of gel. Wet membrane in dH2O and then 20X SSC. Blot O.N.
5. Disassemble blot set-up. UV crosslink DNA to membrane.
6. Place in bag. Add 10 ml of hybridization soln.

3X SSC
0.1% SDS
5X Denhardt's
50% formamide

100 ug/ml sheared calf thymus DNA (Boil for 5 min.)

Mix well and add to bag. Seal bag with as few bubbles as possible.
7. Incubate at 42oC for approx. 3 hrs.
8. Boil a million cpm/ml of radioactively labeled probe for 5 min. Add to blot with a syringe. Seal off hole from syringe and mix by rolling with pipet. Incubate 42oC O.N.
9. Wash blot twice in 2X SSC, 0.1% SDS at r.t. and twice in 0.2X SSC, 0.1% SDS at 55oC.
10. Air dry. Cover with saran wrap and place on film O.N.

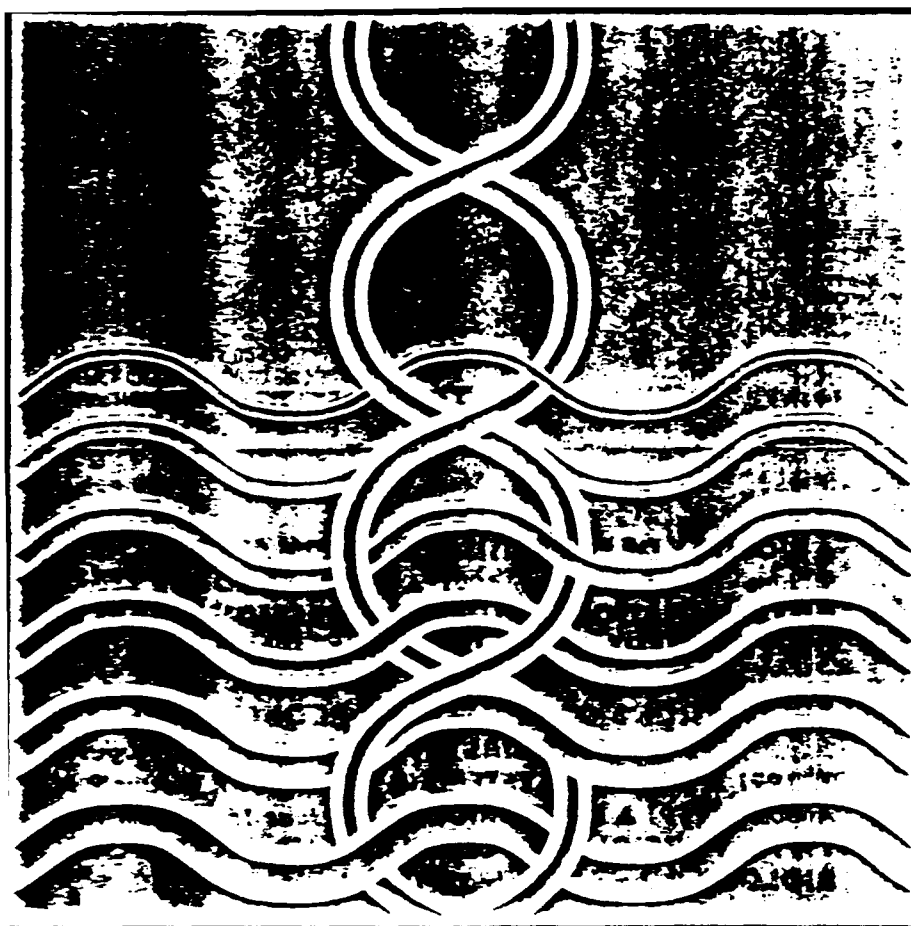
GENECLEAN

When DNA is contained in a gel fragment, include the procedures within ().

1. Add 3 volumes of NaI stock soln. (Incubate 5 min. at 45-55°C to dissolve gel)
2. Add 5 ul of GLASSMILK suspension. Incubate for 5 min.
3. Spin for 5 seconds. Remove supernatant and save.
4. Wash pellet 3 times with NEW WASH.
5. Elute DNA into 7 ul dH₂O.

GENERATION OF ssDNA BY EXONUCLEASE DIGESTION

1. Generate PCR product using one kinased and one unkinased primer.
2. Isolate PCR product.
3. Treat with 8 units of lambda exonuclease (BRL) in 50 ul of 67 mM glycine-NaOH (pH 9.4), 2.5 mM MgCl₂ buffer for 30 min. at 37°C.
4. Extract with phenol/chloroform then chloroform. Ethanol precipitate and resuspend in 7 ul of dH₂O.



APPLIED BIOSYSTEMS

1 single column DNA synthesizer

BECKMAN

1 LS 3801 scintillation counter

2 microfuges

1 GPR w/assortment of adapters

1 new floor model ultracentrifuge w/Ti70.1, SW28, SW60Ti, Vti80, SW40, SW55, & new NVT rotors

1 J2-21 refrigerated centrifuge w/JA-14, JA-20, JS-13 rotors & new adapters for 15ml, & 50ml conical tubes

1 heat sealer

BIOS

4 horizontal gel systems - standard

4 southern blotting gel systems

BRINKMANN

1 model 5415C Eppendorf microfuge

COY LAB PRODUCTS

2 thermal cyclers (PCRs)

FOTODYNE

1 UV Transilluminator (Fotodyne UV 300)

1 Transilluminator Polaroid Camera (Fotodyne PCR-10) w/5-5343 camera hood

IBI

2 STS 45 sequencing apparatuses w/wedge spacers, shark tooth combs, & glass plates

4 CPS power supplies

3 VCV gel systems

ISCO

4 model 452 power supplies